

Short Research Article

Title: Considerable low frequency of soil-transmitted helminths and higher frequency of intestinal protozoa in shanty towns of Libreville, Gabon

ABSTRACT

Aims: Intestinal parasitic infections (IPIs) are a significant public health problem in sub-Saharan Africa. Establishment of effective control strategies such as mass drug administration of antihelminthics are implementing while recent local data in urban areas are lacking. The aim of this study was to assess the frequency of IPIs and coparasitism in patients from shanty towns of Libreville.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Parasitology Mycology at the Université des Sciences de la Santé, Libreville, from February to April 2014.

Methods: Stool samples collected from 101 individuals, aged between 13 and 74 years old, were analysed by direct microscopic examination, merthiolate-iodine-formol concentration and coproculture. Age, education level, type of house and existence of latrine were also recorded.

Results: Among analysed stools, 75.2% (n=75/101) were infected with at least one parasite. Protozoa represented 94.3% of the detected parasites with *Blastocystis(B.) hominis* being the predominant species (41.6%; n=42/101). *Entamoeba(E.) histolytica/dispar* (6.9%; n=7/101) and *Giardia(G.) intestinalis* (1.97%; n=2/101) were the less common protozoa. Soil transmitted helminths (STH) were found in 7.6% (n=8/101) of stool examined, *Trichirus(T.) trichiura* (5.9%) was the most common helminth detected, followed by *Ascaris(A.) lumbricoides* (2.0%), hookworm (1.0%) and *Strongyloides(S.) stercoralis* (1.0%). Coparasitism was present in 47.4% (n=36/76) of positive cases. Within the multi-infected samples, dual and triple infections accounted for 66.7% and 19.4%, respectively. *B.hominis* was the most implicated in co-infections. The use of latrine tended to be associated with STHs carriage ($p=0.06$).

26 **Conclusion:** This study reports a high frequency of intestinal parasites infection in shanty towns of
27 Libreville. An unexpected higher prevalence of protozoa compared to STH is also noticed.

28 INTRODUCTION

29 Intestinal parasitic infections (IPIs) due to soil-transmitted helminth (STH) and protozoa infections
30 have been recognized as neglected tropical diseases (NTDs) and as one of the most significant causes
31 of illnesses among disadvantaged communities from SubSaharan Africa [1,2]. In 2010, it was
32 estimated that IPIs result in 400 to 800 million illness cases [3]. These infections are ubiquitous with
33 high prevalence among the poor and socioeconomically deprived communities who undergo
34 overcrowding, poor environmental sanitation, low level of education and lack of access to safe
35 water, warm climates and adequate moisture, lack of personal or environmental hygiene, sanitation,
36 and education, walking barefoot, and poor health or nutritional status [4]. These factors increase the
37 risk of being contaminated, trapping exposed populations in a perennial cycle of poverty and
38 destitution [1,5]. IPIs identification is important, since some are due to pathogenic organism and,
39 others considered up to now as non-pathogenic, seem to induce intestinal and allergic symptoms
40 that can be chronic. Indeed, STH are recognized as pathogens inducing clinical symptoms while the
41 pathogenic role of protozoan, other than *Entamoeba (E). histolytica* and *Giardia (G.) intestinalis* such
42 as *Blastocystis (B.) hominis*, remains either controversial or less investigated [6]. Previous studies
43 reported that *B. hominis* is probably the most common human gut protozoan in the world, with
44 >50% of prevalence in developing countries but the relationship between its presence and the
45 clinical digestive symptoms is controversial [6,7].

46 Urban migration leads to the creation of precarious urban settlements, with a growth process of
47 shantytowns characterized by poor housing conditions, narrow streets that are not paved, a lack of
48 sanitation, clean water supply and electricity, as well as a poor accessibility to health services. This
49 phenomenon occurs in Libreville, the capital city of Gabon where more than 60% of the total
50 population lives, with a high proportion living in shanty towns. Data on intestinal parasitism are

scarce in this city. Those available focus on STH and were recorded more than 8 years ago in rural areas while since several years, access to safe water is difficult due to frequent supply interruptions. People are supplied in common water sources, such as pumps and then transport the collected water to their home and store it in tanks. Thus, the risk of human and/or waterborne transmission of IPIs would be actually high. Moreover, national deworming programmes are being prepared for country implementation by national control programs. They consist of mass administration of albendazole and praziquantel highly effective against STH whom prevalence estimates remain unknown. It is therefore essential to identify the real burden and reservoirs of these parasites, to facilitate the establishment of effective control strategies. Furthermore, while these programs focus on preschool-aged and school-aged children from semi-urban and rural areas, important gaps regarding IPIs among adults exist. During the weekly consultation performed at the Department of Parasitology Mycology (DPM), patients with symptoms such as pruritus and or abdominal pain are routinely screened for blood and IPIs. The present study was designed to estimate the frequency of IPIs in patients from shanty towns of Libreville consulting at the DPM during a 4 months period.

PATIENTS AND METHODS

Study site

This was a prospective and analytical study performed at the Department of Parasitology Mycology (DPM) of the Faculty of medicine of Libreville, the capital city of Gabon from February to April 2014.

Study population and procedures

Patients seen during the weekly consultations performed at the DPM of the Faculty of Medicine were included if they met the following criteria : providing a stool sample, living in an identified shanty town, to have not changed their place of residence for more than six months, having available parasitological test results, to give oral informed consent for data use. The demographic, socioeconomic and sanitary information were collected by trained field researchers, through semi-

75 structured questionnaires handed to the volunteers. All collected samples were analysed
76 prospectively by routinely used standard methods as previously described: direct microscopic
77 examination of a small amount of stool mixed in a saline solution drop (9‰ sodium chloride),
78 merthiolate-iodine-formaldehyde concentration (MIFc) of eggs, cysts and vegetative forms according
79 to Sapero and Lawless [8]. This method consists of concentrating approximately 1gram of fecal
80 sample crashed in the MIF solution and stained with lugol. Parasite coproculture based on the
81 Harada and Mori technique was performed for the detection of helminth larva [9]. Two trained
82 laboratory technicians undertook the examination using optical microscopes with objectives of 10x
83 and 40x. The result was considered positive if eggs or larvae (for helminths) or cysts/vegetative
84 forms (for protozoa) were found by at least one method. They were considered negative if the entire
85 slide or pellet did not reveal any parasite.

86 **Ethical considerations**

87 All tests performed were part of the routine diagnosis of IPIs in the DPM. Oral informed consent for
88 the use of data was obtained from each participant after the study had been explained. Results were
89 reported within three days and positive patients were seen by the physician and treated according
90 to the national recommendation. Data analysis was performed in agreement with the Gabonese
91 Ministry of Health.

92 **Statistical analysis**

93 Data collected were entered into an Excel spreadsheet and analysed using the Stata® version 12.1
94 software (StataCorp LP, Texas, USA). The statistical tests performed included the Pearson's Chi-
95 square and Fischer's exact for group comparisons. Statistical significance was set at $p < 0.05$.

96 **RESULTS**

97 Data from 101 individuals with a median age of 40[24-54] years were analysed. The majority (n=75,
98 74.3%) had secondary school level, 35 (34.7%) lived in houses made of wood or sheet metal and 48

(47.2%) used latrines. Overall, 26 patients presented clinical symptoms, 10 (9.9%) had only digestive signs (abdominal pain and or diarrhea) and 15 (14.9%) had pruritus as a single sign (n=13) or associated with digestive symptoms (n=2).

IPIs were detected in 76 (75.2%) stool samples with a predominance of protozoa among infected ones (n=76/76; 100.0%) (Table 1). Helminth infections were always associated with a protozoa in positive samples (Table 1 and 2). The most commonly found parasite was *B. hominis* followed by *E.coli* and *Endolimax nanus* (Table 2). *T.trichiura* (5.9%) was the more frequently detected STH species whereas larva of hookworm and *Strongyloides (S.)stercoralis* were rarely present (Table 2). *B.hominis* was more often found in single or coparasitized samples. Patients with polyparasitism carried two to five different species (Table 1). The rate of infected samples with pathogenic protozoa including *B.hominis* was higher than that of non-pathogenic species (n=42/75; 56.0 % versus n=2(/75; 33.3%; $p<0.01$). *B.hominis* was the only parasite found in samples from 11 symptomatic patients. There was no difference in the frequency of IPIs by sex. Coparasitism rate (27.8%; n=25/90) was almost twofold lower among adults compared to children (54.5%; n=6/11) ($p=0.14$). STHs were more frequently found among patients who used latrines (17.4%) compared to patients with conventional toilets (3.8%) ($p=0.06$); while no difference was observed with pathogen protozoan carriage (55.2% versus 61.5% in case of toilets use; $p=0.4$). All individuals who lived in hood house had an IPI.

DISCUSSION

IPIs mapping either as symptomatic or asymptomatic carriage is essential for the design or the improvement or MDA strategies. National deworming programmes were recently included in the package of strategies for the control of endemic parasitic neglected diseases in Gabon. Thus, data should be generated and available for the national control program. However, accurate data are lacking in the country, with only those from rural areas or very old being publically available [10,11]. This study showed a very high prevalence of IPIs in individuals from shanty towns of Libreville.

Although data were not provided by a community based project, 75 out of the 101 patients did not present any digestive symptoms, thus they could represent a subset of the parasite reservoir living in shanty towns, providing important information for public health actions.

The frequency of IPIs is high compared to previous data from Lambarene, a rural city located almost 300km from Libreville and from elsewhere [11-13]. This difference is likely due to the concomitant detection of helminths and protozoa. Indeed, studies previously performed in the country focused on helminth species. The methodology used could also partly explain the most relevant finding of this work which is the predominance of protozoa, more precisely of pathogenic ones in contrast with the frequency of STH infections, in agreement with data from Ethiopia and Rwanda, but in contrast with others [14,15]. Furthermore, such high prevalence of IPIs is observed in a population composed by 80% adults. Adults represent a non-negligible parasite reservoir, thus, epidemiological surveys on IPIs should not only include children, but all age populations.

B.hominis was the most predominant species. It was found as a monoinfection in 11 out of the 26 symptomatic patients. Its pathogenic role is being highlighted by several authors. Its association with clinical sign such as urticarial, abdominal pain, irritable bowel syndrome and diarrhoea in the absence of another aetiology was already demonstrated [16-17]. Screening of *B.hominis* should be envisaged in symptomatic patients, as well as during epidemiological studies.

The frequency of *E.histolytica* and *G.intestinalis* was relatively low, in accordance with data obtained in other areas of the country and outside Africa. However, 4 out the 7 stools infected with *E.histolytica* contained both cysts and vegetative forms. Considering that molecular analysis often discriminate *E.histolytica* among collected cysts, actions against this pathogenic amoeba should be envisaged for therapeutic or preventive management [18].

Compared to *A.lumbricoides* and hookworm, *T.trichiura* was the most frequently detected STH. Its prevalence is high with an estimation 464.6 million people infected worldwide, mainly in sub-Saharan Africa and Asia [1]. This higher rate could also be attributed to technics used: MIFc requires

a small amount of stool compared to the Kato Katz method which is usually used for STH detection in the field; *T.trichiura* parasite density is always high in infected samples; thus the probability of its detection with methods using smaller amount of stool is higher in contrast to other STHs which diagnosis with the Kato Katz is more sensitive in case of low parasite load. Another explanation is the common self-medication practice with albendazole or mebendazole in Libreville, both drugs do not have 100% efficacy against *T.trichiura* [19-20].

Although this study was not aimed at analysing epidemiological factors associated with IPIs carriage, a trend toward an association between STH carriage and non-conventional latrine use, as well as with age was observed in agreement with reports from others [5,13,15].

This study has some limits. It was not community-based, the sample size was small, the intensity of isolates was not determined because the Kato-Katz technique was not employed (it is not used for routine analysis at the DPM).

CONCLUSION

Intestinal parasitic infections are highly prevalent and should be considered as major public health concerns among the poor and socioeconomically communities living in shanty towns. Further studies should be performed to confirm the predominance of protozoa infections among IPIs. If the low frequency of STH is confirmed, the design of actual deworming programmes which are based on antihelminthic mass administration should be revised.

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Table 1 : Profile of intestinal parasitism

PARASITE CARRIAGE	N	%
Helminths	8	7.9
Protozoa	76	75.2
Monoparasitism	40	39.6
Coparasitism	36	35.6
Protozoa + helminths	8	7.9
2 parasites	24	66.7
3 parasites	7	19.4
4 parasites	4	11.1
5 parasites	1	2.8

240 **Table 2 : Prevalence of parasite species (n ; %) detected in the study population**

241

242	SPECIES	TOTAL		MONOPARASITISM		COPARASITISM	
		N	%	n	%	n	%
243							
244	Helminths						
245	<i>Ascaris lumbricoides</i>	2	2.0	0	0.0	2	100.0
246	<i>Hookworm</i>	1	1.0	0	0.0	1	100.0
247	<i>Strongyloides stercoralis</i>	1	1.0	0	0.0	1	100.0
248	<i>Trichirus trichiura</i>	6	5.9	0	0.0	6	100.0
249	Protozoa						
250	<i>Blastocystis hominis</i>	42	41.6	17	40.5	25	59.5
251	<i>Endolimax nanus</i>	28	27.7	8	28.6	20	71.4
252	<i>Entamoaba coli</i>	33	32.7	12	36.4	21	63.6
253	<i>Entamoeba hartmanni</i>	7	6.9	1	14.3	6	85.7
254	<i>Entamoeba histolytica/dispar</i>	7	6.9	1	14.3	6	85.7
255	<i>Giardia intestinalis</i>	2	1.97	1	50.0	1	50.0